Claims

1. (Currently Amended) A method for generating a vector for conditional knockout of a gene in a cell, comprising

using homologous recombination to insert a nucleic acid encoding a first selectable marker flanked by a pair of first recombining sites into a first site in a gene in a bacterial artificial chromosome, wherein a vector comprises the bacterial artificial chromosome;

excising the nucleic acid encoding the selectable maker with a first recombinase specific for the first recombining sites, wherein a single first recombining site remains in the gene;

using homologous recombination to insert a nucleic acid encoding a second selectable marker flanked by a pair of second recombining sites into a second site in the gene; and

excising the nucleic acid encoding the second selectable marker with a second recombinase specific for the second recombining sites, wherein recombination of the recombining sites produces a nucleic acid sequence that cannot be transcribed to produce a functional protein,

thereby generating the vector for conditional knockout of the gene in the cell, wherein the cell is a bacterial cell that comprises a de-repressible promoter operably linked to a nucleic acid encoding Beta and Exo, and wherein using homologous recombination comprises de-repressing the de-repressible promoter, thereby inducing the expression of Beta and Exo.

2. (Canceled).

3. (Previously Presented) The method of claim 1, wherein either the first recombining sites or the second recombining sites comprise a LoxP site.

4. (Previously Presented) The method of claim 1, wherein the first recombining sites comprise a LoxP site.

5. (Previously Presented) The method of claim 1, wherein the second recombining sites comprise a LoxP site.

6. (Previously Presented) The method of claim 1, wherein using homologous recombination to insert the nucleic acid encoding the selectable marker flanked by the pair of first recombining sites comprises

introducing a double-stranded vector comprising the nucleic acid encoding the selectable marker flanked by the pair of first recombining sites into a host cell comprising a nucleic acid sequence encoding Exo, Beta and Gam, operably linked to a de-repressible promoter, wherein the vector further comprises a sufficient number of nucleotides homologous to the bacterial artificial chromosome flanking each of the pair of first recombining sites to achieve homologous recombination;

selecting a host cell in which homologous recombination has occurred.

7. (Previously Presented) The method of claim 1, wherein the cell further comprises an inducible promoter operably linked to a nucleic acid encoding the first recombinase, and wherein excising the nucleic acid encoding the selectable maker comprises inducing the expression of the first recombinase.

8. (Original) The method of claim 7, wherein the first recombinase is Cre.

9. (Original) The method of claim 7, wherein the first recombinase is Flpe.

10. (Original) The method of claim 7, wherein the cell is a bacterial cell.

11. (Canceled).

12. (Previously Presented) The method of claim 1, wherein the cell comprises an inducible promoter operably linked to a nucleic acid encoding the first recombinase, and wherein the first recombination site is the same as the second recombination site.

13. (Original) The method of claim 1, wherein the selectable marker confers resistance of the cell to an antibiotic.

14-21. (Canceled).

22. (Previously Presented) The method of claim 1, wherein the de-repressible promoter is pL.

23. (Previously Presented) The method of claim 6, wherein the de-repressible promoter is pL.

24-26. (Canceled).

27. (New) The method of claim 4, wherein the first selectable marker is neomycin resistance, operably linked to a PGK-EM7 promoter.

28. (New) A method for generating a vector for conditional knockout of a gene, comprising

using homologous recombination in a bacterial cell to insert a nucleic acid encoding a first selectable marker operably linked to a PGK-EM7 promoter flanked by a pair of LoxP sites into a first site in a gene in a bacterial artificial chromosome, wherein a vector comprises the bacterial artificial chromosome, and wherein the bacterial cell comprises a de-repressible promoter operably linked to a nucleic acid encoding Beta and Exo, and wherein using homologous recombination comprises de-repressing the de-repressible promoter, thereby inducing the expression of Beta and Exo;

excising the nucleic acid encoding the selectable maker with Cre recombinase, resulting in a single LoxP site remaining in the bacterial artificial chromosome;

using homologous recombination in the bacterial cell to insert a nucleic acid molecule comprising a FRT site 3' and 5' of a nucleic acid encoding a second selectable marker operably linked

to PGK-FM7 promoter, wherein the nucleic acid molecule further comprises a LoxP site 3' of the 3' FRT site; and

excising the nucleic acid encoding the second selectable marker with FLP, resulting in a second LoxP site and a FRT site remaining in the bacterial articifical chromomose,

thereby generating the vector for conditional knockout of the gene.

29. (New) The method of claim 1, wherein using homologous recombination to insert a nucleic acid encoding a first selectable marker flanked by a pair of first recombining sites into a first site in a gene in a bacterial artificial chromosome comprises the use of a construct comprising 100 to 300 base pairs homologous to the first site in a gene flanking the pair of first recombining sites.